CRYOTISSUE KIT

Protocol

Cryotissue Method

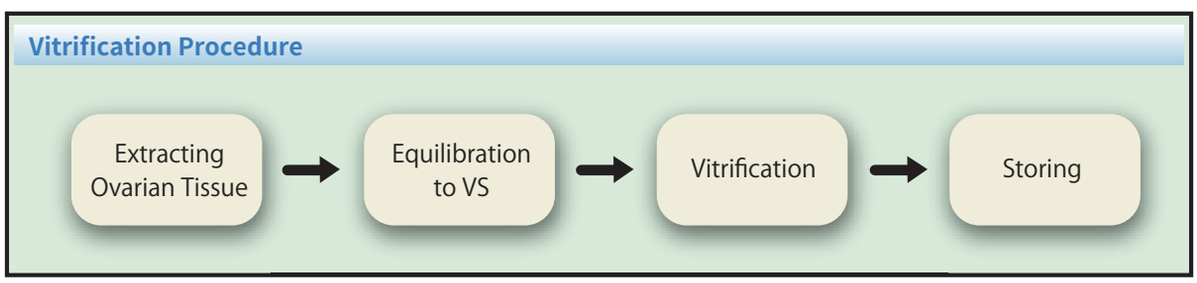
KITAZATO®

Vitrification

Products Used

Cryotissue Vitrification Kit (Ref. VT301-CT)

- Cryotissue X 2
- Square Measure
- Vitrification Media：No.1 Equilibration Solution (ES): 1 X 15ml vial
  No. 2 Vitrification Solution (VS): 1 X 15ml vial



Materials Required

1. Cryotissue Vitrification Kit (Ref. VT 301- CT)
   - Cryotissue X 2
   - Square Measure
   - Vitrification Media
     No.1 Equilibration Solution (ES): 1 X 15ml vial
     No.2 Vitrification Solution (VS): 1 X 15ml vial
2. Rack Cooling-Blue box for liquid nitrogen (Ref. VT-CLB)
3. Liquid Nitrogen (Sterilization is available with PTFE, H020A047A, Advantec)
4. Stopwatch or Timer with count up function
5. Tweezers X 2 (For handling ovarian tissue and cooling)
6. Cane (C-5, My Science Co., Ltd.)
7. Surgical Knife (Replacement Blade: No. 11)
8. Microtome Blade (S35, Feather)
9. Trimming Knife・Handle (F-80 Mini, Feather)
10. 60mm Dish X 2 (1000-060, IWAKI)
11. Sterile Gauze

STEP 1 Preparation

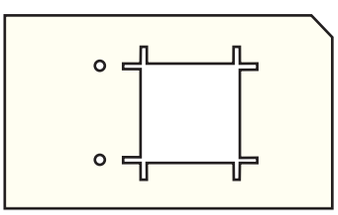
- Write necessary information about a patient on the handle of Cryotissue (See Figure 1).
- Bring ES and VS to room temperature (25~27℃).

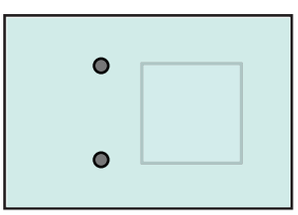
Figure 1

CAUTION Be sure to bring ES and VS to room temperature (25~27℃) to get a high survival rate after thawing. If it is hard to keep the room temperature at 25～27℃ , follow the procedure on P6.

STEP 2 Slicing Ovarian Tissue

Square Measure is a template for cutting ovarian tissue into small slices. It consists of two parts.

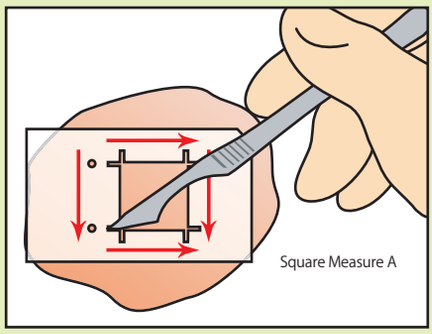

Square Measure A

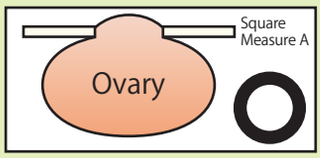

Square Measure B

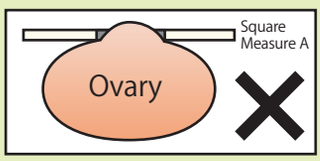
1. Wash an extracted ovary with saline to take off excess blood.
2. Wipe the excess saline on the surface of ovary using a sterile gauze to prevent slipping of Square Measure.
3. Place Square Measure A (with a square hole and a stopper) on the surface of the ovary.
4. The stopper of Square Measure A should be on the left and back side like the picture below. Cut the ovary deeper than 1mm tracing the inner edge of the Square Measure A with surgical knife.

TIP

Start cutting the ovary from the corner of the Square Measure A like the picture below in order to cut it in a complete square. The tissue will come up through the plate after cutting.







5. Carefully place Square Measure B on the Square Measure A and hold it down (See Figure 2).

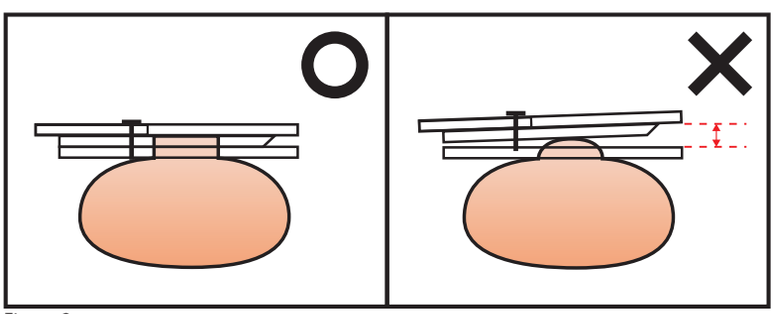


Figure 2

6. Insert a Microtome Blade between the Square Measure A and Square Measure B. Cut into the tissue along the Square Measure A until reaching the stopper. (See Figure 3)

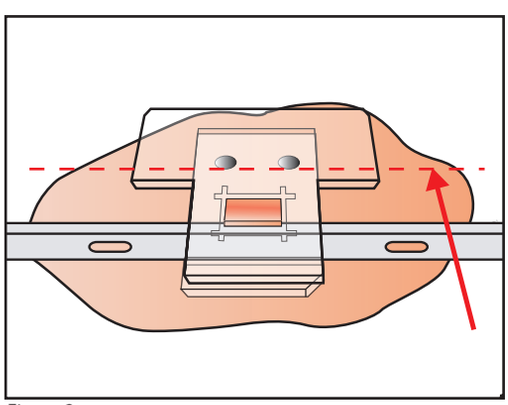
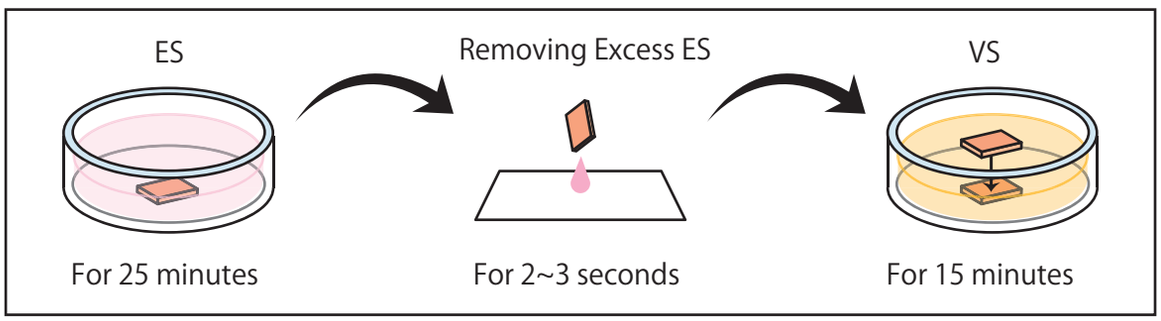


Figure 3

STEP 3 Equilibration to Vitrification Media

※ If it is hard to keep room temperature at 25～27℃ , follow the procedure on P. 6.



1. Pour the full contents of ES vial (15ml) into a 60mm dish. Place the extracted tissue on the dish and wait for 25 minutes (See figure 4).

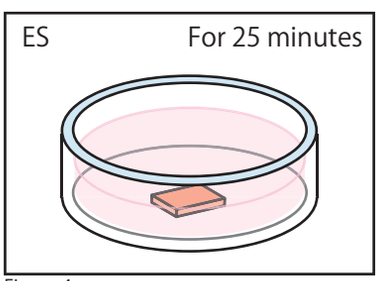


Figure 4

2. Pour the full contents of VS vial (15ml) into a 60mm dish. Transfer the tissue in ES to the surface of VS using tweezers. Right before transferring the tissue to VS, remove the excess ES with a sterile gauze to minimize ES (See figure 5).

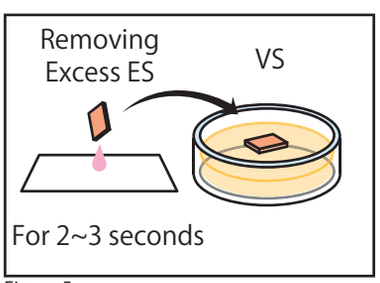


Figure 5

3. Wait for 15minutes. The equilibration is completed if the tissue on the surface of VS free-falls completely (See figure 6).

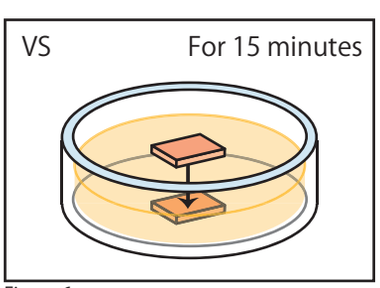


Figure 6

CAUTION In case that the tissue does not free-fall after 15 minutes, wait until the tissue free-falls completely. If the tissue free-falls within 15 minutes, leave it in the VS for at least 15 minutes.

Vitrification

《In case the room temperature is low.》

Keep at 26℃ for the following procedures.

1. Pour warm water at 26℃ to a thermos and warm ES vial to 26℃ in it.

2. Put the extracted tissue in ES vial warmed at 26℃ (See figure 7).

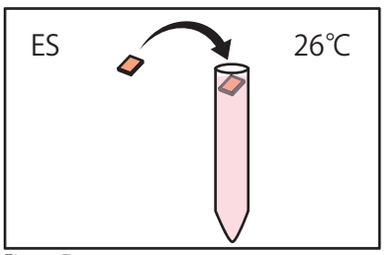


Figure 7

3. Drive a screw cap of ES vial and transfer it to the thermos kept at 26℃ . Wait for 25 minutes (See figure 8).

4. Warm VS vial to 26℃ in the thermos.

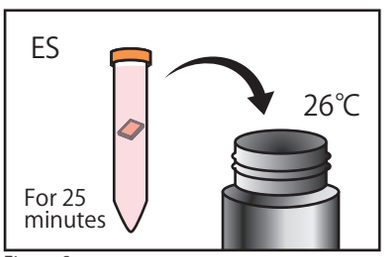


Figure 8

5. After 25 minutes, pour the full contents of ES vial (15ml) with the tissue into a 60mm dish. Transfer the tissue in ES to the surface of VS vial using tweezers. Right before transferring the tissue to VS, remove the excess ES using a sterile gauze to minimize ES (See figure 9).

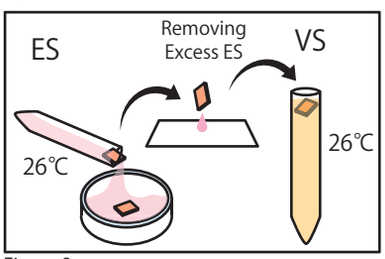


Figure 9

6. Drive a screw cap of VS vial and transfer it to the thermos kept at 26℃ . Wait for 15 minutes (See figure 10).

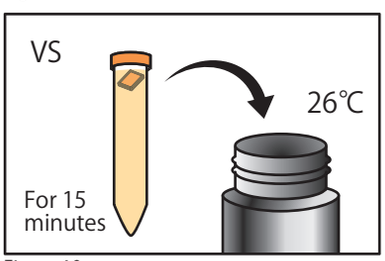


Figure 10

7. The equilibration is completed if the tissue on the surface of VS free-falls completely (See figure 11).

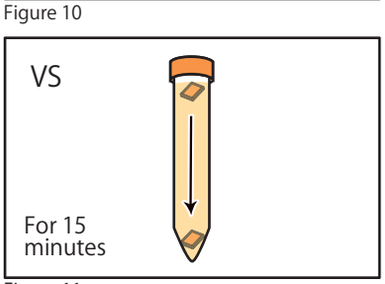


Figure 11

CAUTION

In case that the tissue does not free-fall after 15 minutes, wait until the tissue free-falls completely. If the tissue free-falls within 15 minutes, leave it in the VS for at least 15 minutes.

STEP 4 Vitrification

1. After the equilibration to VS, place the tissue on the Cryotissue (See figure 12).

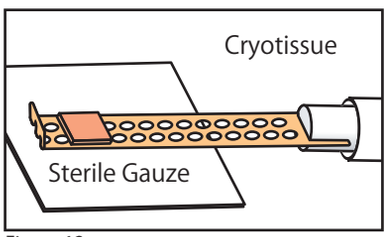


Figure 12

2. Put a sterile gauze to the back of the Cryotissue to remove the excess VS.

3. Plunge the Cryotissue into fresh liquid nitrogen quickly (See figure 13).

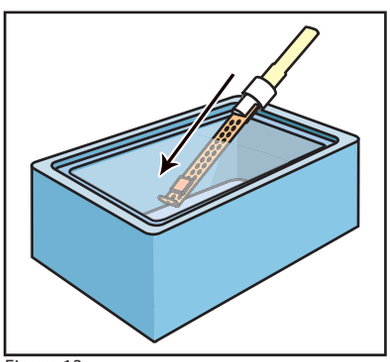


Figure 13

4. Check whether the tissue is translucent. Translucent tissue means vitrified (See figure 14).

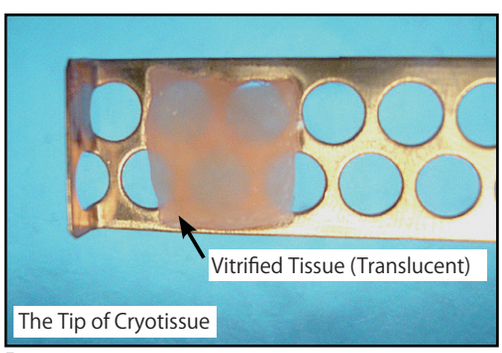


Figure 14

5. Insert the Cryotissue into the cap and twist it. Make sure if it is completely sealed. Store it in the liquid nitrogen using a storage tank (See figure 15).

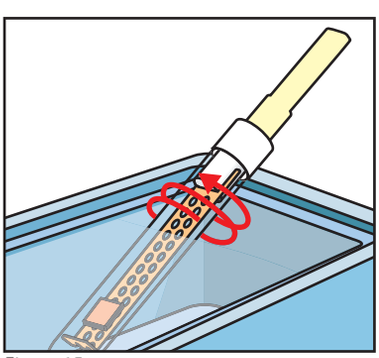


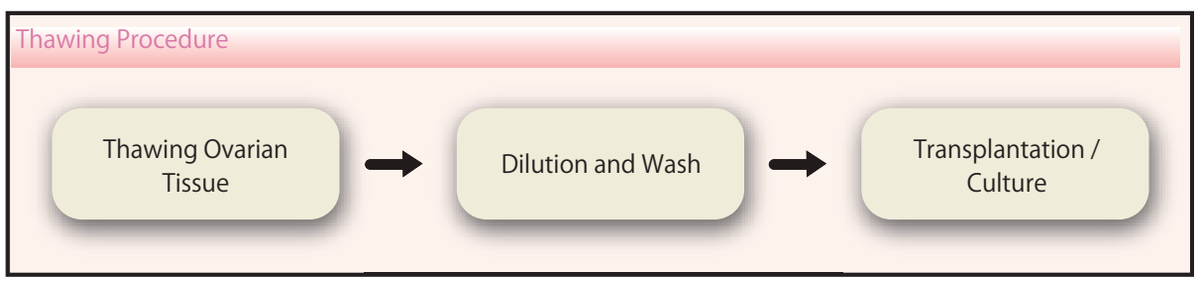
Figure 15

Thawing

Products Used

Cryotissue Thawing Kit (Ref. VT302-CT)

・Thawing Media：No. 1 Thawing Solution (TS): 1 X 45ml vial
No. 2 Diluent Solution (DS): 1 X 15ml vial
No. 3 Washing Solution 1 (WS1): 1 X 15ml vial
No. 4 Washing Solution 2 (WS2): 1 X 15ml vial



Material Required

1. Cryotissue Thawing Kit (Ref. VT302-CT)
   ・Thawing media
   No.1 Thawing Solution (TS): 1 X 45ml vial
   No.2 Diluent Solution (DS): 1 X 15ml vial
   No.3 Washing Solution 1 (WS1): 1 X 15ml vial
   No.4 Washing Solution 2 (WS2): 1 X 15ml vial
2. Rack Cooling-Blue box for liquid nitrogen (Ref. VT-CLB)
3. Liquid Nitrogen (Sterilization is available with PTFE, H020A047A, Advantec)
4. Stopwatch or Timer with count up function
5. Tweezers X 2 (For handling ovarian tissue and cooling)
6. 60mm Dish X 3 (1000-060, IWAKI)
7. 90mm Dish (SH-15-S, TERUMO)

STEP 1 Preparation

Warm TS vial to 37℃ and bring DS, WS1 and WS2 to room temperature (25～27℃).

STEP 2 Thawing

1. Take the cap off of the Cryotissue in liquid nitrogen (See figure 1).

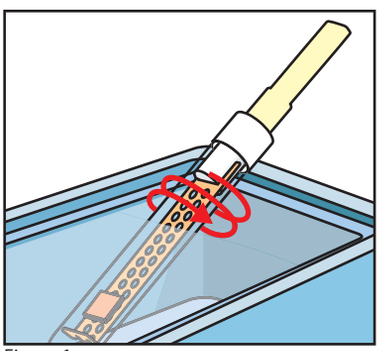


Figure 1

2. Quickly immerse Cryotissue into TS warmed to 37℃ . It should be within 1 second (See figure 2).

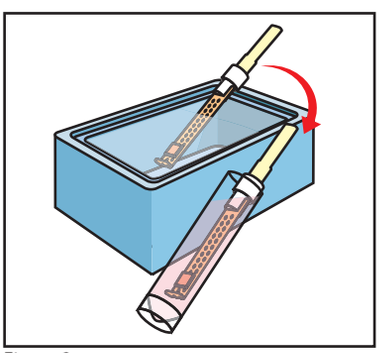


Figure 2

3. Take out the Cryotissue after the tissue comes off by itself. Leave the tissue in TS for 1 minute after immersing (See figure 3).

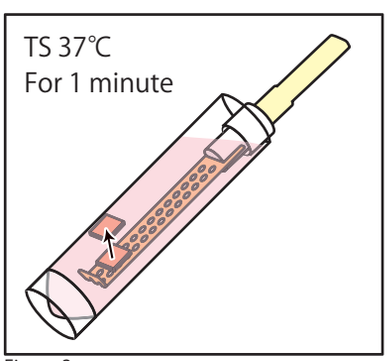
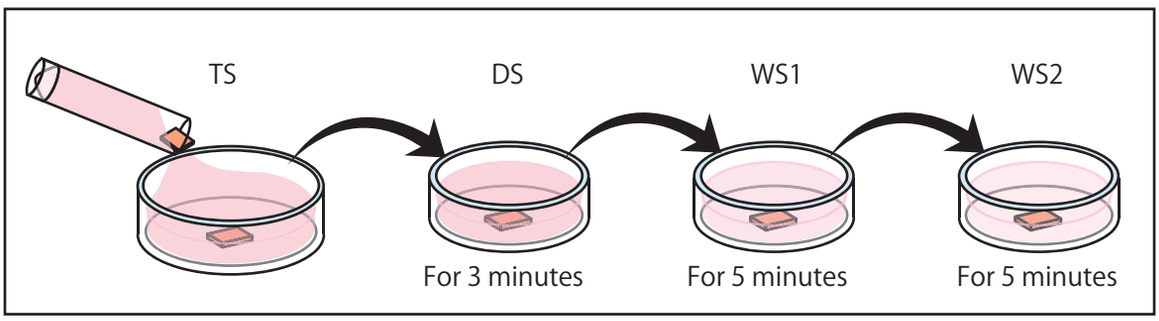


Figure 3

STEP 3 Dilution and Wash



1. Pour the full contents of DS (15ml) into a 60mm dish (See figure 4).

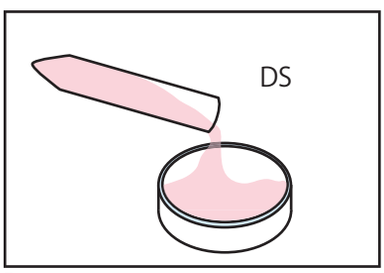


Figure 4

2. Pour the full contents of TS with the tissue into a 90mm dish (See figure 5).

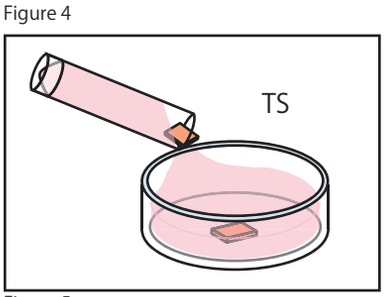


Figure 5

3. Transfer the tissue in TS to DS using tweezers.

4. Wait for 3 minutes (See figure 6).

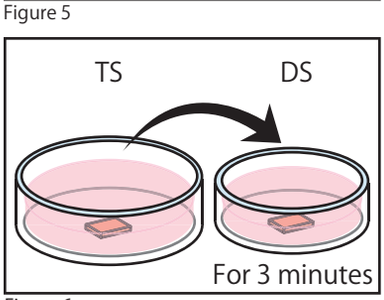


Figure 6

5. Pour the full contents of WS1 (15ml) and WS2 (15ml) into 60mm dishes. Do this preparation while waiting for Dilution is done.

6. Transfer the tissue in DS to WS1.

7. Wait for 5 minutes (See figure 7).

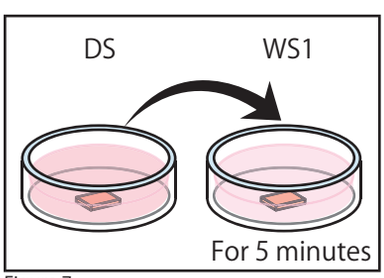


Figure 7

8. Transfer the tissue in WS1 to WS2.

9. Wait for 5 minutes (See figure 8).

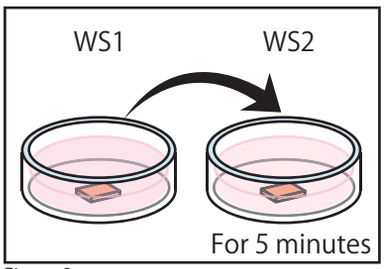


Figure 8

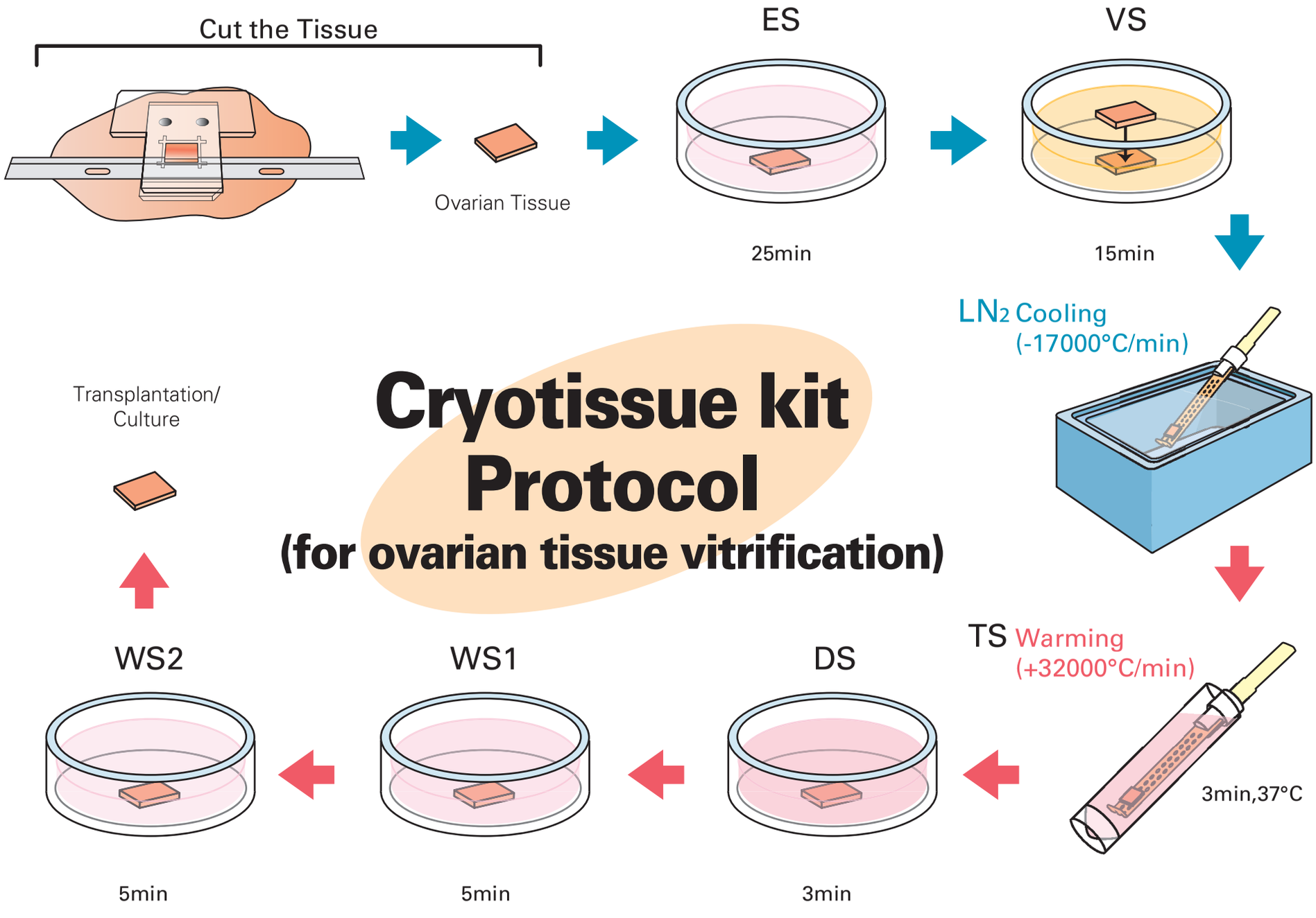
STEP 4 Transplantation / Culture

After 5 minutes in WS2, immediately transplant or culture the tissues.

For your questions,
email to: trading@kitazato-biopharma.com

Cryotissue kit Protocol

(for ovarian tissue vitrification)



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